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13. ABSTRACT ***(Maximum 200 Words)***
The purpose of this research program is to contribute towards two major objectives in support of advancing our ability to prevent or treat bone failure or fragility:

1. Developing and characterizing methods of evaluating bone properties in animal models that goes beyond measures of bone density and global mechanical properties.
2. Evaluating the influence of physical forces and nutritional status on bone biomechanical integrity.

Specifically, it was the purpose of this study to apply a hierarchical approach to quantifying the properties of murine bone to the level of the extracellular matrix. Furthermore, the study was designed to test hypotheses concerning the interplay between vitamin D and calcium nutritional support and physical forces.

Progress during the first year of study has followed the proposed statement of work. All of the mechanical fixtures have been fabricated and calibrated. This included a custom designed and fabricated treadmill system for exercising the animals, as well as whole bone and microspecimen mechanical testing fixtures. The first cohorts of animals have been entered into the study as scheduled and the bones from the first group of animals are beginning to be characterized. Finally, a database structure has been created and scheduled for continuing cohorts of animals prescribed.

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A. Introduction

It is well known that the maintenance and adaptation of bone integrity is dependent on a complex interaction of metabolic and environmental factors (mechanical stresses, nutritional status). Unfortunately, the specific relationships between these factors and the biomechanical competence of bone tissue remains incompletely quantified. As a result, strategies for preventing or effectively treating bone fragility or enhancing general bone health are far from being optimized. The specific goals of this research program is to contribute to two major objectives in support of reducing the incidence of fracture:

a. The development and application of microimaging and testing techniques in animal models to study bone structure function properties.
b. Exploring the influence of calcium and vitamin D metabolism and physical forces on bone integrity.

B. Body

The progress of this research program is described below, as a function of the statements of work that were approved by the USAMRMC for the first year. The statement of work was proposed as follows:

1, The acquisition of DBP founder mice and breeding will be performed during year 1 and 2 to produce 180 animals for testing.

2, Mechanical fabrication and calibration of all testing holders and test fixtures will be completed during the first nine months of study. Maintenance, recalibration and replacement of parts will continue years 2 through 4.

3, Micro CT, whole bone testing of DBP mice will be completed years 1 and 2.

4. Microspecimen production and testing of DBP bones will be completed years 2 through 3.

5. Micro CT, Whole bone testing of C57BL/56 and C3H/HeJ bones will be conducted years 1 through 3.

6. Microspecimen testing of C57BL/6J and C3H/HeJ bone will be tested years 2.5 through year 3.5.

7. Raman imaging, SEM, and light microscopy of DBP mice bone will be conducted in years 1 to 3.

8. Raman imaging, SEM, and light microscopy of C57BL/J6 and C3H/HeJ bone will be tested year 2 through 3.5.

9. Final data analyses and correlations across all mice and groups will be completed during year 4.

Since most of the tasks were described as objectives to be completed over 1 to 3 years, the progress report can't follow these 9 tasks precisely. We have presented the specific tasks that were proposed for completion during the first year. The tasks are outlined in **"bold"** followed by a description of the accomplishments.

Mechanical fabrication and calibration of all testing holders and test fixtures will be completed during the first nine months of study.

1. Treadmills and exercise protocol

The initial plan was to use a forced running wheel. In exploring the use of the wheels, it became clear that the mice tended to hop instead of run, cling to the walls or slide to avoid running. To avoid these problems and implement a more reliable system to apply physical loads, we designed, fabricated and calibrated a treadmill system. The systems were modeled from a commercially available instrument from Columbus Instruments (rodent treadmill). Performing the design and fabrication in our laboratory enabled us to have a custom optimized design and save substantial costs. The treadmill is illustrated below.

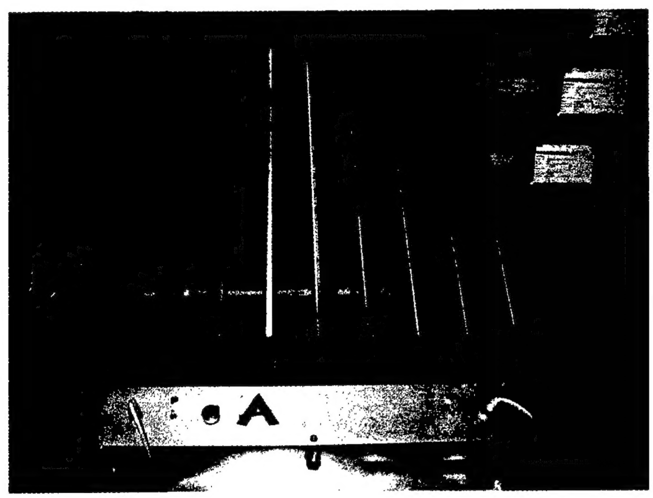

Table 1: Exercise Protocol

Week	Days	Speed (m/min)	Duration (min)
0	3-5	8	10-15
1	1-2	10	15
1	3-5	10	18
2	1-2	12	18
2	3-5	12	22
3	1-2	14	22
3	3-5	14	26
4	1-2	17	26
4	3-5	17	30
5-8	1-5	17	30

Two treadmills were fabricated and calibrated. Each treadmill is capable of exercising up to eight mice in individual lanes. The user can adjust the running speed from 4 to 35 m/min and the running surface can be inclined from -15° to 15 ° above horizontal. A stimulus can be created using electrical shock grids to encourage the mice to run. The intensity of the stimulus is user controlled and ranges from 0 - 150 volts. These treadmills are used five days a week for approximately 2 hours/day. The protocol used for exercising is shown in Table 1 and was based on the exercise regime of a previous study (Kohut 2001). In the

protocol, 'Days' refers to successive exercising days (ex. 1-2 = Mon & Tues; 3-5 = Wed – Fri.) This particular protocol allows for training up to a speed of 17 m/min for 30 minutes. In order to feasibly manage the large cohort of mice over the duration of the research program, the mice are divided such that there will be 9 week periods where two groups of 24 mice will be exercised following the protocol, with the groups being staggered by one week.

Exercise Protocol:

The mice are placed on the treadmill in their respective lanes with the belt still and shock grids on. The next step is to start the belt at 10m/min, start the timer, and ensure the mice are running. After each minute, the speed of the treadmill is increased by 1m/min until the desired speed is reached. At this point, the mice are run until the predetermined amount of time has elapsed. Then the belt and shock grids are deactivated and the mice are returned to their cages. If at any point during the experiment a mouse becomes exhausted, the mouse must be removed and allowed time to rest.

Exhaustion:

We have developed guidelines to determine when a mouse should be removed from the treadmill due to exhaustion based on experience and consultation with laboratory animal medicine veterinarians. The guidelines are:

a. Being shocked for greater than 5 consecutive seconds on the shock grid without attempting to reengage the treadmill.
b. Continuously falling back onto the shock grid.
c. Sustaining an injury that would hinder further exercise.

2. Development and fabrication of the mechanical testing fixtures and systems.

The devices and protocols for both the four-point bending of the femurs (Figure 2) and the vertebral compression testing (Figure 3) have been developed and tested. Various mechanical and material properties will be determined from these tests. Both systems function by utilizing the devices within the testing frame of an MTS Bionix servo-hydraulic testing system. A Matlab program was written for analysis and is fully functioning.

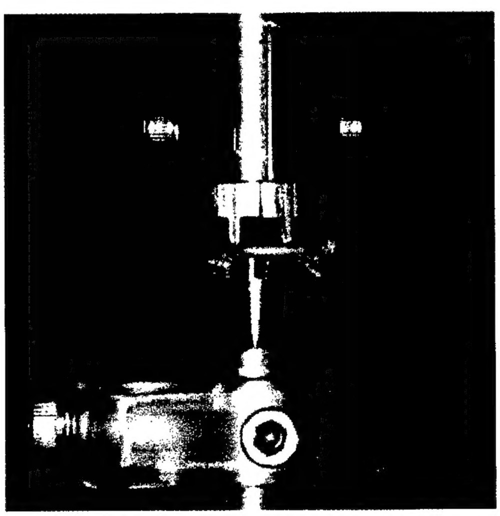

Figure 2: Four-point bending fixtures to test of a mouse femur.

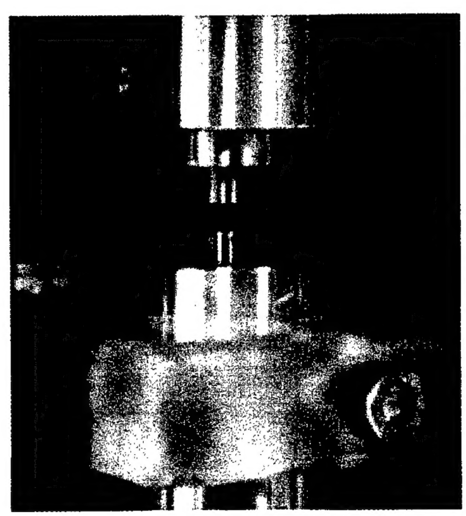

Figure 3: Compression system to test a mouse vertebra.

Acquire and begin to evaluate the DBP mice for evaluating the effects of vitamin D and physical exercise on bone properties.

DBP breeder mice (4 females and 2 males) have been obtained from Dr. Nancy Cooke at the University of Pennsylvania. These mice are being used to generate our own population of heterozygote breeders. Due to background noise, homozygous breeders cannot be used. We have also developed protocols for genotyping the newborn mice. We expect to have sufficient experimental animals to enter the vitamin D cohorts within the next 6 months

Acquire and begin to evaluate the C3H/HeJ and C57BL/6J mice to evaluate the effects of calcium metabolism and physical exercise on bone properties.

The C3H/HeJ and the C57BL/6J mice are ordered through Jackson Laboratory. The mice have been numbered in increments of 7 starting with the number 2 (2, 9, 16, 23, etc.) to help avoid clerical errors. Currently 144 of the 360 mice have already been entered in the study and the first 48 were sacrificed on 9/10/02. The second cohort of 48 mice was sacrificed on 10/30/02. A new cohort of 48 mice has already been acquired and is currently going through the exercise protocols. A page from our database of animals is illustrated below to demonstrate how the experimental design is incorporated in the record. Note the numbering scheme that increments by 7 for each animal.

Mouse ID	Cage ID	Group	Mark	Sex	Strain	DOB	Diet	Exercise
674	25a	3a	R	M	C57		Low	Yes
681	25b		L	M	C57		Low	Yes
688	25c		B	M	C57		Low	Yes
695	25d		N	M	C57		Low	Yes
702	26	3b	R	M	C57		Low	No
709	26		L	M	C57		Low	No
716	26		B	M	C57		Low	No
723	26		N	M	C57		Low	No
730	27	3a	R	F	C57		Norm	Yes
737	27		L	F	C57		Norm	Yes
744	27		B	F	C57		Norm	Yes
751	27		N	F	C57		Norm	Yes
758	28	3b	R	F	C57		Norm	No
765	28		L	F	C57		Norm	No
772	28		B	F	C57		Norm	No
779	28		N	F	C57		Norm	No
786	29a	3a	R	M	C57		High	Yes
793	29b		L	M	C57		High	Yes

800	29c		B	M	C57		High	Yes
807	29d		N	M	C57		High	Yes
814	30	3b	R	M	C57		High	No
821	30		L	M	C57		High	No
828	30		B	M	C57		High	No
835	30		N	M	C57		High	No
842	31	4a	R	F	C57		Low	Yes
849	31		L	F	C57		Low	Yes
856	31		B	F	C57		Low	Yes
863	31		N	F	C57		Low	Yes
870	32	4b	R	F	C57		Low	No
877	32		L	F	C57		Low	No
884	32		B	F	C57		Low	No
891	32		N	F	C57		Low	No
898	33a	4a	R	M	C57		Norm	Yes
905	33b		L	M	C57		Norm	Yes
912	33c		B	M	C57		Norm	Yes
919	33d		N	M	C57		Norm	Yes
926	34	4b	R	M	C57		Norm	No
933	34		L	M	C57		Norm	No
940	34		B	M	C57		Norm	No
947	34		N	M	C57		Norm	No
954	35	4a	R	F	C57		High	Yes
961	35		L	F	C57		High	Yes
968	35		B	F	C57		High	Yes
975	35		N	F	C57		High	Yes
982	36	4b	R	F	C57		High	No
989	36		L	F	C57		High	No
996	36		B	F	C57		High	No
1003	36		N	F	C57		High	No
1010	37a	4a	R	F	C57		High	Yes
1017	37b		L	F	C57		High	Yes

Sacrifice Procedures and Additional Protocol

Although we didn't propose to conduct studies for hematology and serum chemistry analysis in our initial grant application, in hindsight, after completion of the first round of exercise and nutrition modulation studies, we believe that it would be valuable to examine these parameters. It is well established that exercise can affect hematologic parameters. For instance, exercise increases red cell volume and hemoglobin concentration. In addition, it is important that we determine the general health of the treatment animals relative to controls. Although we have taken great care to appropriately acclimate the mice to exercise, it is possible that the exercise may cause an increased level of stress. Alterations in general health will be indicated by changes in white blood cell and red blood cell parameters. This is obviously an important consideration for the interpretation of our final results because alterations in general health may adversely impact bone remodeling. In addition to the measurement of hematologic parameters, it is also prudent to study a number of serum-based indices. Specifically, we propose to measure Ca, P, Vitamin D, osteocalcin, and the resorption marker NTx. The dietary treatments, as well as exercise, could affect serum mineral concentrations as well as parameters of bone remodeling (osteocalcin an NTx). As a result, we have modified our protocols at sacrifice to take a blood sample from each of the mice. This procedure requires a chamber induction with 5% isoflurane gas with 1% oxygen anesthesia. The anesthesia is maintained with a mask at 2% isoflurane with 1% oxygen (for 10-15 minutes). When a surgical plane of anesthesia has been achieved (as determined by muscle relaxation, lack of a conscious response to superficial pain, and absence of a palpebral reflex) a pasteur pipette is used to collect 250 microliters of blood by retro-orbital bleeding. The blood is collected into an EDTA tube, centrifuged, and plasma frozen at –70 pending analysis. Following bleeding, the mouse is then sacrificed via cervical dislocation. This is an AVMA approved method for mice and the mice are anesthetized while cervical dislocation is performed. Bilateral pneumothorax is preformed to ensure death. The mice are then frozen and the bones extracted for testing as needed. The right femur and the 8^{th} caudal vertebrae will then be micro-CT scanned. From these scans, geometric features, such as the femoral cross sectional area and the vertebral trabecular number, can be determined. In addition, four-point bending will be performed on the right femurs and the vertebrae will be compression tested to collect mechanical data on the bones.

C. Key Research Accomplishments

- Designed and fabricated exercise treadmills
- Implemented exercise protocol and developed guidelines for exhaustion of mice
- Designed, fabricated and calibrated mechanical testing hardware and software.
- Developed and implemented database management tools for entire program
- Acquired and in progress of breeding DBP mice
- Completed protocols for first 96 animals in calcium/exercise groups and all are sacrificed and evaluation of bone properties is underway
- Completed schedule and have already entered next cohort of 48 animals into calcium and exercise groups
- Developed new protocol for blood acquisition at sacrifice to monitor metabolic and bone turnover parameters; implemented for first cohort of sacrificed animals

D. Reportable Outcomes

No abstracts or publications have been completed to date. Study has just ended first year and first groups of animals are presently under evaluation.

E. Conclusions

The first year of work has been extremely successful and we have accomplished all of our proposed tasks and are slightly ahead of schedule. It should be noted that the breeding of the DBP mice has been more difficult than anticipated and we are looking for ways to accelerate that process in order to stay on schedule for that study cohort. The design and use of the two treadmills had gone extremely well and we expect to have our first bone structure/function data within the next 3 months.

F. References

Kohut, ML, Boehm, GW, Moynihan, JA 2001 Moderate exercise is associated with enhanced antigen-specific cytokine, but not IgM antibody production is aged mice. Mech Ageing Dev 122:1135-1150.

G. Appendices

We have included a printout of our databases for the animals entered into the study.

A1: Rpt Mouse Info: This demonstrates our main database in Access and the animals entered into the exercise study to date.

A2: Food consumption database output record.

A3: Schedule tables for entering next several cohorts for calcium study that demonstrates how the timetables are developed.

Rpt_Mouse_Info

mouse_id	cageid	sex	dob	diet	strain	exercise	group_number
2	1	M	4/21/2002	Low	C57	☑	1
9	1	M	4/21/2002	Low	C57	☑	1
16	1	M	4/21/2002	Low	C57	☑	1
23	1	M	4/21/2002	Low	C57	☑	1
30	2	M	4/21/2002	Low	C57	☐	1
37	2	M	4/21/2002	Low	C57	☐	1
44	2	M	4/21/2002	Low	C57	☐	1
51	2	M	4/21/2002	Low	C57	☐	1
58	3	F	4/21/2002	Normal	C57	☑	1
65	3	F	4/21/2002	Normal	C57	☑	1
72	3	F	4/21/2002	Normal	C57	☑	1
79	3	F	4/21/2002	Normal	C57	☑	1
86	4	F	4/21/2002	Normal	C57	☐	1
93	4	F	4/21/2002	Normal	C57	☐	1
100	4	F	4/21/2002	Normal	C57	☐	1
107	4	F	4/21/2002	Normal	C57	☐	1
114	5	M	4/21/2002	High	C57	☑	1
121	5	M	4/21/2002	High	C57	☑	1
128	5	M	4/21/2002	High	C57	☑	1
135	5	M	4/21/2002	High	C57	☑	1
142	6	M	4/21/2002	High	C57	☐	1
149	6	M	4/21/2002	High	C57	☐	1
156	6	M	4/21/2002	High	C57	☐	1
163	6	M	4/21/2002	High	C57	☐	1
170	7	M	4/21/2002	Low	C57	☑	1
177	7	M	4/21/2002	Low	C57	☑	1
184	7	M	4/21/2002	Low	C57	☑	1
191	7	M	4/21/2002	Low	C57	☑	1

mouse_id	cageid	sex	dob	diet	strain	exercise	group_number
198	8	M	4/21/2002	Low	C57	☐	1
205	8	M	4/21/2002	Low	C57	☐	1
212	8	M	4/21/2002	Low	C57	☐	1
219	8	M	4/21/2002	Low	C57	☐	1
226	9	F	4/21/2002	Normal	C3H	☑	1
233	9	F	4/21/2002	Normal	C3H	☑	1
240	9	F	4/21/2002	Normal	C3H	☑	1
247	9	F	4/21/2002	Normal	C3H	☑	1
254	10	F	4/21/2002	Normal	C3H	☐	1
261	10	F	4/21/2002	Normal	C3H	☐	1
268	10	F	4/21/2002	Normal	C3H	☐	1
275	10	F	4/21/2002	Normal	C3H	☐	1
282	11	M	4/21/2002	High	C3H	☑	1
289	11	M	4/21/2002	High	C3H	☑	1
296	11	M	4/21/2002	High	C3H	☑	1
303	11	M	4/21/2002	High	C3H	☑	1
310	12	M	4/21/2002	High	C3H	☐	1
317	12	M	4/21/2002	High	C3H	☐	1
324	12	M	4/21/2002	High	C3H	☐	1
331	12	M	4/21/2002	High	C3H	☐	1
338	13	F	4/28/2002	Low	C57	☑	2
345	13	F	4/28/2002	Low	C57	☑	2
352	13	F	4/28/2002	Low	C57	☑	2
359	13	F	4/28/2002	Low	C57	☑	2
366	14	F	4/28/2002	Low	C57	☐	2
373	14	F	4/28/2002	Low	C57	☐	2
380	14	F	4/28/2002	Low	C57	☐	2
387	14	F	4/28/2002	Low	C57	☐	2
394	15	M	4/28/2002	Normal	C57	☑	2
401	15	M	4/28/2002	Normal	C57	☑	2

mouse_id	*cageid*	*sex*	*dob*	*diet*	*strain*	*exercise*	*group_number*
408	15	M	4/28/2002	Normal	C57	☑	2
415	15	M	4/28/2002	Normal	C57	☑	2
422	16	M	4/28/2002	Normal	C57	☐	2
429	16	M	4/28/2002	Normal	C57	☐	2
436	16	M	4/28/2002	Normal	C57	☐	2
443	16	M	4/28/2028	Normal	C57	☐	2
450	17	F	4/28/2002	High	C57	☑	2
457	17	F	4/28/2002	High	C57	☑	2
464	17	F	4/28/2002	High	C57	☑	2
471	17	F	4/28/2002	High	C57	☑	2
478	18	F	4/28/2002	High	C57	☐	2
485	18	F	4/28/2002	High	C57	☐	2
492	18	F	4/28/2002	High	C57	☐	2
499	18	F	4/28/2002	High	C57	☐	2
506	19	F	4/28/2002	Low	C3H	☑	2
513	19	F	4/28/2002	Low	C3H	☑	2
520	19	F	4/28/2002	Low	C3H	☑	2
527	19	F	4/28/2002	Low	C3H	☑	2
534	20	F	4/28/2002	Low	C3H	☐	2
541	20	F	4/28/2002	Low	C3H	☐	2
548	20	F	4/28/2002	Low	C3H	☐	2
555	20	F	4/28/2002	Low	C3H	☐	2
562	21	M	4/28/2002	Normal	C3H	☑	2
569	21	M	4/28/2002	Normal	C3H	☑	2
576	21	M	4/28/2002	Normal	C3H	☑	2
583	21	M	4/28/2002	Normal	C3H	☑	2
590	22	M	4/28/2002	Normal	C3H	☐	2
597	22	M	4/28/2002	Normal	C3H	☐	2
604	22	M	4/28/2002	Normal	C3H	☐	2
611	22	M	4/28/2002	Normal	C3H	☐	2

mouse_id	cageid	sex	dob	diet	strain	exercise	group_number
618	23	F	4/28/2002	High	C3H	☑	2
625	23	F	4/28/2002	High	C3H	☑	2
632	23	F	4/28/2002	High	C3H	☑	2
639	23	F	4/28/2002	High	C3H	☑	2
646	24	F	4/28/2002	High	C3H	☐	2
653	24	F	4/28/2002	High	C3H	☐	2
660	24	F	4/28/2002	High	C3H	☐	2
667	24	F	4/28/2002	High	C3H	☐	2

Appendix 2

Cage ID	Group ID	Week	Date of leftover	Starting Amount (g)	Leftover amount (g)	Consump. by cage	# mice in cage	avg comsum./mouse	Special
1	1	1	19-Jul-02	119.7	48.2	71.5	4	17.875	
2		1		121.4	36.2	85.2	4	21.3	
3		1		118.2	61.7	56.5	4	14.125	
4		1		116.8	60.5	56.3	4	14.075	
5		1		120.4	55.3	65.1	4	16.275	
6		1		117.9	41.6	76.3	4	19.075	
7		1		119.4	37.5	81.9	4	20.475	
8		1		120.2	38	82.2	4	20.55	
9		1		116.9	62.6	54.3	4	13.575	
10		1		117.3	51.3	66	4	16.5	
11		1		119.7	21.5	98.2	4	24.55	
12		1		116.3	22.6	93.7	4	23.425	
1	1	2	26-Jul-02	141.3	41.8	99.5	4	24.875	
2		2		141.1	14.8	126.3	4	31.575	
3		2		139.1	60.7	78.4	4	19.6	
4		2		138.8	51.1	87.7	4	21.925	Cage '5' =
5a		2		36.3	5.7	30.6	1	30.6	32.1
5c		2		36.8	3.2	33.6	1	33.6	
6		2		139.3	41.6	97.7	4	24.425	
7		2		140	45.5	94.5	4	23.625	
8		2		139.6	40.8	98.8	4	24.7	
9		2		105.9	35.5	70.4	3	23.46666667	
10		2		140.6	56.5	84.1	4	21.025	
11		2		140.9	38.4	102.5	4	25.625	
12		2		140.4	35.5	104.9	4	26.225	
1a	1	3	2-Aug-02	35.1	6.5	28.6	1	28.6	Cage '1' =
1b		3		35.8	10.1	25.7	1	25.7	25.825
1c		3		35.1	7.8	27.3	1	27.3	
1d		3		35.7	14	21.7	1	21.7	
2		3		141.2	27.5	113.7	4	28.425	
3		3		141.7	63.4	78.3	4	19.575	
4		3		140.3	58.8	81.5	4	20.375	Cage '5' =
5a		3		37.1	11.6	25.5	1	25.5	27.9
5c		3		37.6	7.3	30.3	1	30.3	
6		3		140.4	45.4	95	4	23.75	
7		3		141.2	46.6	94.6	4	23.65	
8		3		139.3	42.2	97.1	4	24.275	
9		3		103.6	33.9	69.7	3	23.23333333	
10		3		139.3	57.1	82.2	4	20.55	
11		3		140.4	47.4	93	4	23.25	
12		3		141.8	33.6	108.2	4	27.05	

Cage ID	Group ID	Week	Date of leftover	Starting Amount (g)	Leftover amount (g)	Consump. by cage	# mice in cage	avg comsum./mouse	Special
1a	1	4	9-Aug-02	36.2	8	28.2	1	28.2	
1b		4		35.7	10.3	25.4	1	25.4	
1c		4		34.4	7.4	27	1	27	Cage 1 =
1d		4		33.9	11.9	22	1	22	25.65
2		4		139.7	34	105.7	4	26.425	
3		4		139.2	55.9	83.3	4	20.825	
4		4		141.2	55.9	85.3	4	21.325	
5a		4		35.7	7.8	27.9	1	27.9	Cage 5 =
5c		4		36.1	5.3	30.8	1	30.8	29.35
6		4		140.9	43.4	97.5	4	24.375	
7		4		141.2	49.1	92.1	4	23.025	
8		4		140	52.3	87.7	4	21.925	
9		4		105.9	33.3	72.6	1	72.6	
10		4		140.6	53.7	86.9	4	21.725	
11		4		139.4	43.9	95.5	4	23.875	
12		4		142.3	34.8	107.5	4	26.875	
13	2	1	9-Aug-02	141.5	60.3	81.2	4	20.3	
14		1		139.4	58.8	80.6	4	20.15	
15a		1		35.2	5	30.2	1	30.2	Cage 15 =
15b		1		36.6	9.8	26.8	1	26.8	28.35
15c		1		34.1	7	27.1	1	27.1	
15d		1		36.4	7.1	29.3	1	29.3	
16		1		140.3	33.9	106.4	4	26.6	
17		1		141.6	51.8	89.8	4	22.45	
18		1		142.1	50	92.1	4	23.025	
19		1		142.1	55	87.1	4	21.775	
20		1		141.2	51.8	89.4	4	22.35	
21		1		139.4	32.6	106.8	4	26.7	
22		1		138.2	26.2	112	4	28	
23		1		142.3	47.3	95	4	23.75	
24		1		142.9	38	104.9	4	26.225	
1a	1	5	16-Aug-02	35.1	9	26.1	1	26.1	
1b		5		39.3	15	24.3	1	24.3	Cage 1 =
1c		5		36.8	11.9	24.9	1	24.9	24.775
1d		5		35.4	11.6	23.8	1	23.8	
2		5		140.5	31.5	109	4	27.25	
3		5		139.9	54.2	85.7	4	21.425	
4		5		141.5	54	87.5	4	21.875	
5a		5		35.7	8	27.7	1	27.7	Cage 5 =
5c		5		37.6	3	34.6	1	34.6	31.15
6		5		141.7	43.6	98.1	4	24.525	
7		5		140.4	54.2	86.2	4	21.55	
8		5		143.1	67.1	76	4	19	
9		5		107	35.5	71.5	3	23.83333333	
10		5		140	55.9	84.1	4	21.025	
11		5		141	41.8	99.2	4	24.8	
12		5		141.8	34.5	107.3	4	26.825	

Cage ID	Group ID	Week	Date of leftover	Starting Amount (g)	Leftover amount (g)	Consump. by cage	# mice in cage	avg comsum./mouse	Special
13	2	2	16-Aug-02	142.5	55.4	87.1	4	21.775	
14		2		139.5	57.4	82.1	4	20.525	
15a		2		37.5	9.4	28.1	1	28.1	Cage 15 =
15b		2		39.4	12.6	26.8	1	26.8	26.2
15c		2		38.5	15.3	23.2	1	23.2	
15d		2		36.3	9.6	26.7	1	26.7	
16		2		143.6	141.7	1.9	4	0.475	
17		2		140	139	1	4	0.25	
18		2		142.3	139.3	3	4	0.75	
19		2		142.4	61.9	80.5	4	20.125	
20		2		140	55.4	84.6	4	21.15	
21		2		140.7	45.7	95	4	23.75	
22		2		140.7	42.4	98.3	4	24.575	
23		2		141.8	51.7	90.1	4	22.525	
24		2		140.6	44.7	95.9	4	23.975	
1a	1	6	23-Aug-02	35.2	8.7	26.5	1	26.5	Cage 1 =
1b		6		37	11.3	25.7	1	25.7	26.83333333
1c		6		37.2	8.9	28.3	1	28.3	
2		6		141.9	28.8	113.1	4	28.275	
3		6		141.5	53.6	87.9	4	21.975	
4		6		141.4	53.9	87.5	4	21.875	
5c		6		36.3	7.7	28.6	1	28.6	28.6
6		6		142.5	47.1	95.4	4	23.85	
7		6		140.3	39.1	101.2	4	25.3	
8		6		140.3	56.9	83.4	4	20.85	
9		6		106.2	34.7	71.5	3	23.83333333	
10		6		141.5	55.3	86.2	4	21.55	
11		6		139.7	43.4	96.3	4	24.075	
12		6		139.9	32	107.9	4	26.975	
13	2	3	23-Aug-02	139.1	44.8	94.3	4	23.575	
14		3		140.7	49.9	90.8	4	22.7	
15a		3		40.9	13.8	27.1	1	27.1	Cage 15 =
15b		3		36.5	6.9	29.6	1	29.6	26.05
15c		3		37.5	15.1	22.4	1	22.4	
15d		3		37.4	12.3	25.1	1	25.1	
16		3		44	51.3	-7.3	4	-1.825	
17		3		52.5	50.4	2.1	4	0.525	
18		3		45	26.5	18.5	4	4.625	
19		3		141.9	53.8	88.1	4	22.025	
20		3		141.2	55	86.2	4	21.55	
21		3		140.1	56	84.1	4	21.025	
22		3		141.1	48.5	92.6	4	23.15	
23		3		140.1	50.9	89.2	4	22.3	
24		3		140.9	51.5	89.4	4	22.35	

Cage ID	Group ID	Week	Date of leftover	Starting Amount (g)	Leftover amount (g)	Consump. by cage	# mice in cage	avg comsum./mouse	Special
1a	1	7	30-Aug-02	36.2		36.2	1	36.2	Cage 1 =
1b		7		37.1		37.1	1	37.1	36.96666667
1c		7		37.6		37.6	1	37.6	
2		7		141.2		141.2	4	35.3	
3		7		140.4		140.4	4	35.1	
4		7		140.8		140.8	4	35.2	
5c		7		34.9		34.9	1	34.9	34.9
6		7		140.7		140.7	4	35.175	
7		7		139.2		139.2	4	34.8	
8		7		141.2		141.2	4	35.3	
9		7		105.6		105.6	3	35.2	
10		7		141.6		141.6	4	35.4	
11		7		138.8		138.8	4	34.7	
12		7		141		141	4	35.25	
13	2	4	30-Aug-02	139.7		139.7	4	34.925	
14		4		140.8		140.8	4	35.2	
15a		4		36.7		36.7	1	36.7	Cage 15 =
15b		4		37.6		37.6	1	37.6	36.625
15c		4		35.2		35.2	1	35.2	
15d		4		37		37	1	37	
16		4		142.2		142.2	4	35.55	
17		4		139.2		139.2	4	34.8	
18		4		141.9		141.9	4	35.475	
19		4		141.9		141.9	4	35.475	
20		4		140.5		140.5	4	35.125	
21		4		141.6		141.6	4	35.4	
22		4		140.3		140.3	4	35.075	
23		4		143.2		143.2	4	35.8	
24		4		140.6		140.6	4	35.15	

Appendix 3

Strain of Mouse	Diet	Exercise (yes or no)	Male or Female	Period 1 (July 1-Sept 23) Group 1	Group 2	Period 2 (Sept 23 -Dec 9) Group 3	Group 4	Period 3 (Jan 6-March 24) Group 5	Group 6	Period 4 (March 24-June 2) Group 7	Group 8	Period 5 (June 2-Aug 11) Group 9	Group 10	Total
C57BL/6J	low	yes	Male	3	0	3	0	4	0	5	0	0	0	15
	norm	yes	Male	0	4	0	4	0	4	0	3	0	0	15
	high	yes	Male	1	0	6	0	4	0	4	0	0	0	15
C57BL/6J	low	no	Male	4	0	4	0	4	0	3	0	0	0	15
	norm	no	Male	0	4	0	4	0	4	0	3	0	0	15
	high	no	Male	4	0	4	0	4	0	3	0	0	0	15
C57BL/6J	low	yes	Female	0	3	0	4	0	4	0	4	0	0	15
	norm	yes	Female	4	0	4	0	4	0	3	0	0	0	15
	high	yes	Female	0	3	0	4	0	4	0	4	0	0	15
C57BL/6J	low	no	Female	0	4	0	4	0	4	0	3	0	0	15
	norm	no	Female	4	0	4	0	4	0	3	0	0	0	15
	high	no	Female	0	4	0	4	0	4	0	3	0	0	15
C3H/HeJ	low	yes	Male	4	0	0	0	4	0	4	0	3	0	15
	norm	yes	Male	0	4	0	0	0	4	0	4	0	3	15
	high	yes	Male	4	0	0	0	4	0	4	0	3	0	15
C3H/HeJ	low	no	Male	4	0	0	0	4	0	4	0	3	0	15
	norm	no	Male	0	4	0	0	0	4	0	4	0	3	15
	high	no	Male	4	0	0	0	4	0	4	0	3	0	15
C3H/HeJ	low	yes	Female	0	4	0	0	0	4	0	4	0	3	15
	norm	yes	Female	3	0	0	0	4	0	4	0	4	0	15
	high	yes	Female	0	4	0	0	0	4	0	4	0	3	15
C3H/HeJ	low	no	Female	0	4	0	0	0	4	0	4	0	3	15
	norm	no	Female	4	0	0	0	4	0	4	0	3	0	15
	high	no	Female	0	4	0	0	0	4	0	4	0	3	15
				43	46	25	24	48	48	45	44	19	18	360

Strain of Mouse	Diet	Exercise (yes or no)	Male or Female	# Finished with Exercising and Sacrificed	# Currently Exercised	# to be Exercised	# scanned	# analyzed	# mechanical tested
C57BL/6J	low	yes	Male	3	3	9	0	0	0
	norm	yes	Male	4	4	7	0	0	0
	high	yes	Male	1	6	8	0	0	0
C57BL/6J	low	no	Male	4	4	7	0	0	0
	norm	no	Male	4	4	7	0	0	0
	high	no	Male	4	4	7	0	0	0
C57BL/6J	low	yes	Female	3	4	8	0	0	0
	norm	yes	Female	4	4	7	0	0	0
	high	yes	Female	3	4	8	0	0	0
C57BL/6J	low	no	Female	4	4	7	0	0	0
	norm	no	Female	4	4	7	0	0	0
	high	no	Female	4	4	7	0	0	0
C3H/HeJ	low	yes	Male	4	0	11	1	0	0
	norm	yes	Male	4	0	11	3	0	0
	high	yes	Male	4	0	11	2	0	0
C3H/HeJ	low	no	Male	4	0	11	0	0	0
	norm	no	Male	4	0	11	0	0	0
	high	no	Male	4	0	11	2	0	0
C3H/HeJ	low	yes	Female	4	0	11	0	0	0
	norm	yes	Female	3	0	12	3	0	0
	high	yes	Female	4	0	11	0	0	0
C3H/HeJ	low	no	Female	4	0	11	0	0	0
	norm	no	Female	4	0	11	4	0	0
	high	no	Female	4	0	11	0	0	0
total				89	49	222	15	0	0